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A Technique for the Recording of Bioelectric Potentials from Free-flying Insects (Lepidoptera: *Heliconius erato*)[1,2]

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(Plates I & II)

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[For further ecological details of meteorology and biotic zones, see "Introduction to the Ecology of the Arima Valley, Trinidad, B. W. I.," by William Beebe, *Zoologica,* 1952, 37 (13) 157-184.

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Introduction

In spite of extensive and highly imaginative study of the neurological control of insect behavior, many fundamental questions remain unanswered. Even the role of the brain remains a subject of controversy. Roeder (1963) stressed the role of inhibition, while other workers (*e.g.,* Wiersma, 1962) have contended that this effect has been overemphasized.

Even as basic a question as the nature of the control mechanisms responsible for the initiation and maintenance of flight remain unanswered. Weis-Fogh (1956) gave evidence for the purely reflex control of the non-fibrillar, indirect flight muscles of the locust, *Schistocera,* and has been supported by Pringle (1957). More recently, however, Wilson (1961) gave excellent evidence of the central nervous system playing an essential role in supplementing the reflex mechanisms in the same organism.

Both workers agreed that decerebrate animals possessed all the mechanisms necessary for normal flight. That the brain should play no role in such activities is somewhat surprising when one considers that similar basic motor patterns, *e.g.,* walking (Roeder, 1963) and sound production (Huber, 1960), have been shown to be related to protocerebral activity. It seems likely that, at a minimum, such centers must be involved in processing the complex sensory input which arises during flight.

In the past, experiments in this general area have concentrated on "tethered" flight, whereby the organism was firmly mounted, usually by the pterothorax, etc., and then induced to "fly" by eliciting the tarsal reflex (Fraenkel, 1932), sometimes supplemented by an airsteam. Such a situation, while having the advantage of controllability, obviously fails to truly simulate actual flight conditions, as the variations induced by pitch, roll, moving field, etc., have been largely eliminated, thereby minimizing the activity in any feed-back loops that might exist.

Based upon this background, preliminary investigations were undertaken to determine the

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practicability of recording bioelectric potentials from insects permitted to fly with comparative freedom. This note reports the development of a simple technique which has allowed the recording of an "electro-encephalogram" from free-flying butterflies, while simultaneously recording photographically the physical activity of the organism.

Methods and Materials

Heliconius erato adonis, used in these experiments, is a medium-sized (2½" wingspread), black, neotropical butterfly with brilliant scarlet wing patches. It has been the subject of numerous studies including: genetical (Emsley, 1964), behavioral (Crane, 1955), and electrophysiological (Swihart, 1965).

The insects were normally caught in the wild and maintained in large outdoor insectaries until required for experimentation.

The experiments themselves were conducted in a smaller (6′ × 6′ × 6′) insectary which was completely enclosed by aluminum screening. The cage and the electrical equipment were grounded to earth.

The key element in the technique was the extremely fine wire which served as both electrode and lead to amplifier input. Nichrome V alloy wire with enamel insulation, .001" in diameter, manufactured by Driver-Harris Co., was employed. This was found to have a remarkably high degree of tensile strength and flexibility, with a resistance of only 5,000 ohms/foot.

With the butterfly restrained, the stripped and sharpened end of one wire about 4′ in length was placed just beneath the cuticle on the mid-dorsal surface of the head. A similar wire that served as an indifferent electrode was inserted into the dorsal aspect of the thorax or abdomen. Rigid attachment of the electrodes to the cuticle was achieved with a rosin-beeswax cement (Fig. 1). Tangling of the wires was minimized by cementing them together at short intervals with very small drops of UHU cement. The free ends of the wires were fitted with pin jacks. Several butterflies treated in this manner were observed for three to four days after the operation, and showed no apparent ill effects.

The free ends of the wire were connected to the cathode follower input of a Grass P-6 D.C. preamplifier which was operated in the single-ended mode, with the indifferent electrode grounded directly to earth. The input itself was suspended from the center of the roof of the insectary, and consequently the butterfly could fly freely throughout the upper two-thirds of the cage.

The amplified potentials were monitored on a Tektronix 564 oscilloscope and simultaneously fed into the optical sound track of an Auricon Cine-Voice 16 mm. camera (Model CM-72A) equipped with a synchronous motor drive, operating at 24 frames per second.

Results

The recording obtained by this technique did, of course, vary with the position of the band-pass filters of the amplifier. Thus either a high-frequency or low-frequency EEG could be recorded. Any form of mechanical stimulation, such as touching the antennae, abdomen, blowing on the insect, etc., resulted in high frequency, non-synchronous activity, showing considerable after-discharge. Little or no major low-frequency activity accompanied such stimulation (Fig. 2).

On the other hand, as soon as flight was initiated, a well-defined, low-frequency, rhythmic discharge was observed. This consisted of a brief train of spikes (1 to 6), followed by a period of quiet. This pattern repeated itself approximately 17 times per second (Figs. 3, 4). On an average, the quiet period lasted twice as long as the period of activity. Frequently the first several trains, associated with the initiation of flight, contained a higher average number of spikes than were recorded during sustained flight. Thus a typical pattern was 4, 5, 5, 2, 4, 4, 5, 4, 3, 3, 3, 3, 2, 3, 2, etc.

On a number of occasions recordings were obtained from insects that were walking and frequently such activity was accompanied by very slow movements of the wings. Even though the wing movements were of an amplitude quite similar to those made during flight, no low-frequency EEG was detected.

Conclusions

It seems clear that the recorded potentials originated in the supra-esophageal ganglion. Not only was the indifferent electrode carefully grounded to earth, but no detectable difference in the waveform resulted from changes in its location (thorax vs. abdomen). Furthermore, recordings from the thoracic muscles of Lepidoptera show a simple one spike per wingbeat relationship (Roeder, 1951), while recordings from the thoracic ganglia (Pringle, 1957) show about four spikes per wingbeat at regular time intervals. Neither of these patterns is similar to that recorded from the head.

There is, however, a published report of trains of spikes associated with flight mechanisms that is amazingly similar to that observed in the present experiments. Wilson (1961) illustrates the response recorded from nerve IB of *Schistocera,* which carries the output of the wing sense or-

gans. His published records show trains of 2 to 5 spikes occurring at the wingbeat frequency, and separated by periods of quiet twice the duration of the active period. He further notes, "Activity in the sensory unit is greatest at the beginning and end of flight."

In any case, it seems highly unlikely that such a variable pattern of discharges can be associated with the motor neurons of non-fibrillar flight muscles. On the other hand, the failure to detect trains when the organism moves the wings very slowly is consistent with phasic sense organs.

As noted above, Weis-Fogh (1956) attempted to demonstrate the reflex control of flight. Wilson (1961) pointed out that such mechanisms act "on top" of what is determined by the central nervous system. In Wilson's view, however, such determination arose in the thoracic ganglia, since decerebrate animals flew normally.

There is nevertheless some question as to the level at which such determination occurs. Wilson reports that severing the connectives between thoracic ganglia 1 and 2 produced only ambiguous results, while Chadwick (1953) reported that flight movements never occur if the same surgery is performed on *Periplaneta.*

The authors' personal experience with *H. erato* has indicated that even the insertion of a semi-microelectrode into the protocerebrum of an otherwise intact animal can result in a serious impairment of flight ability. When such an organism is thrown into the air, the wings will be moved, but the flight is often only an uncoordinated downward spiral. Such animals may be stimulated to walk and may live for many days but cannot be induced to demonstrate effective flight.

Furthermore, our knowledge of the basic economy of the insect nervous system suggests that we would not detect the activity of the wing sense organs in the vicinity of the protocerebrum, unless that organ was involved in processing this information.

It is well known that in *Schistocera,* wind-sensitive hairs on the head provide an important input relative to flight activity. These are known to discharge directly into the cord. In butterflies there appears to be similar types of organs, *i.e.,* the so called Jordan's organ (Eltringham, 1933). These are regions between the compound eyes which contain many fine hairs, easily displaced by the slightest wind current. The authors have observed that a butterfly flying in tethered flight can be stopped virtually instantly by touching these hairs with a fine camel's hair brush. As opposed to the locust hairs, however, the nerve from this organ is reported to run directly to the protocerebrum.

On the basis of the foregoing discussion, the following conclusions are suggested:

(1) It seems possible that there may exist a whole hierarchy of controls for certain motor patterns, with each succeeding level capable of "refining" the activity of the more peripheral elements. Such a system may extend all the way "up" to the protocerebrum.

(2) The investigation of such a hypothesis can, perhaps, be associated by the utilization of the technique presented in this note, as it would seem to do much in facilitating the analysis of neurological activity under conditions tending to preserve the delicate patterns of sensory input.

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EXPLANATION OF THE PLATES

PLATE I

FIG. 1. *H. erato* with the recording electrode cemented firmly beneath the cuticle of the mid-dorsal portion of the head. The wire was placed beneath the cuticle and then looped through the rosin-beeswax cement so that attachment would be stronger. The picture also shows the indifferent electrode held beneath the cuticle of the dorsal portion of the thorax (far right) and then held by a second drop of cement to insure rigid attachment.

FIG. 2. *H. erato* being stimulated mechanically by touching the abdomen with a pin (**a, i, h, g**) while feeding on *Lantana* flower. The result of such stimulation was high-frequency, non-synchronous activity showing considerable after-discharge. In this figure, as in Fig. 3, the optical tract of the film has been shifted in position to compensate for the normal displacement of the camera's recording head from the photographic image.

PLATE II

FIG. 3. In this sequence, while walking towards a flower taped to the side of the cage, *H. erato* has been stimulated to fly by a flash of light (**a**). Prior to actual flight (**a** through **f**), the optical tract shows only the typical, high-frequency, non-synchronous discharge. However, as free flight commences (**g** through **j**), the pattern is changed to a well-defined pattern of low-frequency, rhythmic discharge. This pattern is repeated twice; between frames **h** and **i**, and toward the end of frame **j**.

FIG. 4. A longer portion of the optical track during a period of free flight. The low-frequency, rhythmic discharge can be observed as consisting of brief trains of spikes. Each activity train is then followed by a period of quiet approximately twice the length of the active period. Spikes may number between 1 and 6 per train, and the pattern repeats itself approximately 17 times per second. This particular sequence lasted ⅓ sec. and shows 5½ trains of 3-4 spikes.